bio-protocol Preprint

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CAT filter assay

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An abbreviated version of this protocol was published in eLIFE in Jul 2016

NaLi-H1: A universal synthetic library of humanized nanobodies providing highly functional antibodies and intrabodies

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Detailed protocol

Chloramphenicol resistance assay
Randomly selected VHHs from naïve library were subcloned into pAOCAT using the NcoI and NotI restriction sites. Chloramphenicol resistance assay was performed using BL21(DE3) cells transformed with the pAOCAT-VHH fusion constructs under the control of T7 promotor, thus expressing nanobodies carboxy-terminaly fused to Choramphenicol Acetyl Transferase cDNA upon IPTG induction. Bacteria were used for inoculating 500 µL of LB containing kanamycin (35 lg/mL) and glucose (0.2%), and were grown at 37 °C until OD600 was 0.8. The cytoplasmic expression of the VHH-CAT-fusion proteins was induced for 2 h by the addition of 0.2 mM IPTG. At the end of the induction period, bacteria were diluted in serial dilutions (10µl + 90µl of PBS), then aliquots of 4 µL of each dilution ach were spotted as replicates on several LB–agar plates containing IPTG (0.1 mM) and increasing chloramphenicol concentrations from 0 to 500 µg/ml. Bacteria were incubated at 30 °C for 20 h before quantification of the colony formation. The resistance level was evaluated according to the colony growth rate at the different chloramphenicol concentrations.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. olichon, a. (2021). CAT filter assay. Bio-protocol Preprint. bio-protocol.org/prep780.
2. Moutel, S., Bery, N., Bernard, V., Keller, L., Lemesre, E., de Marco, A., Ligat, L., Rain, J., Favre, G., Olichon, A. and Perez, F.(2016). NaLi-H1: A universal synthetic library of humanized nanobodies providing highly functional antibodies and intrabodies. eLIFE. DOI: 10.7554/eLife.16228